Mater. Res. Soc. Symp. Proc. Vol. 845 © 2005 Materials Research Society AA8.10

Nanofeaturing materials for specific cell responses

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ABSTRACT

A review of the ways in which cells react to nanofeatured surfaces is given. One of the prime reactions is of adhesion or otherwise to such surfaces. Topography appears to be of considerable importance and a wide range of cell properties are affected by the type, scale and regularity of topography. Chemistry can be combined with topography to fine- tune effects. Mechanical forces are also of importance but in practice it is hard to control these.
Examples will be given of methods of controlling adhesion, morphology, orientation, movement, phagocytic activity and activation and gene expression of cells, Effects vary according cell type and also the spacing and size of nanofeatures. A discussion of the application of these findings to the medical devices concludes this short review.

INTRODUCTION

In the last few years there has been a developing realisation that there are at least three main routes for the phenotypic modification of cells in addition to modifying the genetic background. The three routes are chemical signaling, mechanical signals and topography; some references which establish these three routes are given by [1]
Nanofeaturing implies fabrication and the possibilities are already extensive for producing topography [1,2]. Nanoprinting, as opposed to microprinting, is still a technique in its infancy and microprinting gives too poor edge definition to be considered as a method of obtaining nanomerically defined edges. But fabrication methods are very likely to improve, see [3]. Nanomechanical devices are possible but at the moment probably impractical chiefly because of the difficulty of removing the devices from the body This is in contrast to the chemical signals which are likely to be degraded enzymatically and those of nanotopography which can be made on a biodegradable polymer.

EXPERIMENTAL DETAILS

Fabrication

Precise nanotopography can be made by many techniques but electron beam lithography followed by dry etching to produce a master die or polymer demix systems are widely used [2,4]. Multiple replicas of these surfaces were made by embossing or injection moulding in polycaprolactone or polycarbonate. Cell culture and assessment. Primary cell cultures as well as cell lines were used to investigate the biological effects of these surfaces in a variety of manners, see [5].

RESULTS

Order and random patterns

One important question not yet fully answered is whether nanofeatures either topographic or chemical must be presented to the cell in a non-random manner. Early results suggest that random patterns of topography may have little effect on cells see [6] whereas order, scale and symmetry may be very important [6,7]. The fabrication of ordered chemical features is only just beginning so there is little data available to resolve this question in the chemical field, but see [8]. The extent and accuracy of order required needs to be examined but it is clear that the polymer demixed surfaces used by Dalby and his colleagues [9,10] have clear effects on a variety of cell features even though they are somewhat imperfect, see Fig 1. Nor do we know what the minimum area or length of a piece of nanofeature needs to be to cause a cell to react to it though lengths greater than 1000 nm of a vertical edge of a few nm are needed to produce a reaction. This is the discontinuity effect first noted by Curtis and Clark [12] and studied more fully by the Swedes [13]. In experiments of this type there may be some uncertainty as to what the best control surface is but tissue culture grade polystyrene, bearing random topographic features, is often taken as one type of control and ultra-flat glass or mica surfaces as another suitable control. The results on adhesion from both these surfaces are much the same for both surfaces and much more adhesive than the Nanopitted surfaces and much less adhesive than the nanogrooved surfaces.

Adhesion

Effects on the adhesion of many cells caused by growth on a range of nanotopographies have been reported [5,7,11,13]. The effects vary according to nature of the topography and include pits, pillars, cones, cliffs and pyramids as well as scale and array pattern of the feature. Reactions of different cell types are often very similar though the range of sizes over which they react may vary. There is a vast field open to further investigation here.

Morphology and cytoskeleton

Cell shape and cytoskeletal organization are affected by the type of nanotopography on which the cell has attached and Dalby [5,11-12,14] has provided extensive detail. See also below under Filopodia.

Cell movement

Cell movement is accelerated on the nanogrooved surfaces [15]

Proliferation

Growth on nanofeatured surfaces [14.] increases proliferation (shorter cell cycle time when measured). However, it should be noted that this has been tested on only a few cell types for short culture periods. Apoptosis does not appear to occur,

Gene expression

The work of Dalby [16-17] shows that both precise defined nanotopography and the rather less precise nano-island topography made by polymer demixing) cause considerable changes in gene expression in several cell types. It is clear that the changes follow a sequence of expressions as they do in controls but the sequences are not identical. This area of work opens the possibility of designing structures and scaffolds that will have a degree of control of gene expression. Effects of nanometer sized objects on a surface altered production of the cytokines (IL-6 and IL-8) [10].

Phagocytosis

Macrophages show considerable shape change, and enhanced movement and increased phagocytosis when grown on grooves of 40 nm height [15].

Filopodia

Dalby described [18] enhanced extension of filopodia from several cell types when the filopodia extend over nanopitted or nano-island surface, He suggests that the filopodia are exploring the surface for areas at which to attach.

DISCUSSION

(The majority of results discussed below were described in Results.)

Mechanisms of reaction

The question of macromolecular adsorption being linked to nanotopography is unresolved. But it seems unlikely that differential adsorption of macromolecules from the medium explains the phenomena seen because surfaces of very differing chemistry in a range of different macromolecular species in the media have similar effects on a wide variety of cells.

Mechanisms by which nanofeatures may act are still somewhat unclear. However, the following points should be noted. Cells are exceedingly sensitive to ordered nanotopography and appear to be detecting the dimensions of the surface. Two different explanations can be offered. First, that the redistribution of surface forces in the layer of fluid over the topography due to that topography alters the adhesiveness of the surface. This is consistent with the observed 'superhydrophobic' nature of some of these surfaces. Second, an alternative explanation is that the surface concentrates adhesion in such a way that contractile forces generated by the cells operate to generate in some cases instability in the cell and in others alignment of the cytoskeleton leads in turn to morphological changes see [19] and perhaps to changes in chromosomal packing [20] and thus to changes in gene expression.

Applications

What cell behaviors should be encouraged ?

A simple answer to this is to state that those behaviors (including gene expressions) should be encouraged which will alleviate disease and/or encourage repair and replacement of damaged tissue as well as obviating those events which might lead to apoptosis, lysis or non-adhesion. Examples and suggestions are set out below.

Use of low adhesion surfaces

Examples of this are the use of such surfaces to reduce fouling of devices by cell attachment, eg stents eg implanted electrodes, delivery of fluids in artificial capillaries and narrow tubes for fluid drainage and delivery devices

Requirement for High Adhesion

Groove-ridge nanotopography and also micrometrically wide pits which are nanometrically deep enhance cell adhesion. This, together with the possibility of orienting cells into the best positions mechanically for effective attachment, might be applied to situations such as osseointegration or the integration of soft tissues with other soft tissues eg partial organ grafts into an existing organ. This could also be applied to the surfaces of sensors and prosthetic devices to ensure they remain in long-term contact with the desired organ.

Trapping

Trapping cells in especial positions seems to be possible. There are in principle two ways of doing this. One is to inhibit movement of a cell when it reaches a particular site by means of establishing high adhesion but no clues to orient cell movement the other appears to be one-way gates so that the cell can enter the trap but cannot leave it. If such devices can be made a range of interesting possibilities open up for instance alleviation of inflammation by trapping inflammatory cells from entering the damaged region.

Orientation of intercellular material and of nerves,

It is already clear that cells and cells secreting extracellular materials will tend to align to fibres and to linear nanometric features so it should be possible to guide nerves etc in useful directions. There has been much work on larger scale structures to guide nerves, so-called nerve conduits, but apparently little has been done yet using features at the nanoscale level. Zhu et al [21] obtained good orientation of glioma cells with grooves on polystyrene 210nm periodicity and 30-4onm deep.

Maintaining and repairing tissue and organ boundaries.

Many tissues are bonded by a polarised surface layer of cells that are non-adhesive at the outside but adhesive on their inner sides. Damage to such tissues eg synovia can block the normal function of such parting surfaces which is basically to allow tissues and organs to glide past each other. Other examples of this are the loops of the intestines which must be able to move over each other during peristalsis, the eyelids which must not fuse to each other or to the cornea during sleep. Implantation of a biodegradable polymer sheet with a low adhesion surface will prevent cell attachment to the polymer and provide a physical barrier (of polymer) to stop two tissues fusing. The adhesions that may form after operation or in certain diseases are examples of the failure of processes that should keep tissues separate. Yet tissues must also maintain or establish adhesions during wound repair.

a) Tissue joining or welding

Wocjciak [22] et al showed that epitenon cells in separate explants would migrate along grooved substrate stretching between the explants and then join them up by a continuous cellular bridge. Later a second layer of cells appeared to migrate along the first and in this way successive layers were added to build a multicellular bridge In this way cut ends of a tendon could be welded together. Later this was tested in an in vivo situation. Since tissue welding is very much required to heal wounds this could be a useful approach.

b) Keeping tissue apart

Many tissues are bonded by a polarised surface layer of cells that are non-adhesive at the outside but adhesive on their inner sides. Damage to such tissues eg synovia can block the normal function of such parting surfaces which is basically to allow tissues and organs to glide past each other. Other examples of this are the intestines which must be able to move over each other during peristalsis or the eyelids which must not fuse to each other or to the cornea during sleep. Implantation of a biodegradable polymer sheet with a low adhesion surface will prevent cell attachment to the polymer and provide a physical barrier (of polymer) to stop two tissues fusing. Grooving the sheet may provide cell polarisation. The adhesions that may form after operation or in certain diseases are examples of the failure of processes that should keep tissues separate. An example of such a device at the micron scale is shown in Figure .1 which keeps synovia and epitenon apart but welds tendon ends together.

Figure 1 (above)A typical well-repaired tendon after section and implantation of a polydioxanone (PDS) microgrooved device is shown. Traces of degraded PDS can be seen . Scale bar 80 micron. The device has 'welded' the tendon together and kept the synovia and epitenon from fusing.

Movement

Cell movement rates are enhanced on some dimensions of groove. This in itself is not necessarily a desirable feature but if polarisation (one way migration) can be achieved something really useful for tissue engineering will have been achieved. There are two reports using microtopography which suggest that this might be possible, Dow et al [23] and Boocock [24].

Cell proliferation rate

Effective repair of damaged areas is likely to require an enhanced rate of proliferation during the most active phases of repair followed by a return to normality as repair finishes. Reports suggest that nanotopography can control cell proliferation rates. If this is substantiated for several cell types use might be made of this in wound healing and also in tumour control.

Cell orientation and polarisation

Cells in many tissues are visibly oriented and possibly all are polarised in some feature, for instance secretion in many epithelia. Their orientation may be related to extracellular material orientations or to other cues such as the surface of the organ or other surrounding cells. Microscale and nanoscale topography or microscale printed chemistry can achieve this.

Cytoskeletal orientation and polarisation

Underlying the orientations described above there is a cytoskeletal organization of one or more of the three main systems, actin, tubulin and vimentin. Less studied but still important is the rarer cytoskeletal proteins, cytokeratins (confined to epithelia cells), neurofilaments and desmins. The external nano-or microtopography may act to initiate and maintain cell polarisation

Cell morphology

This is related to orientation and polarisation but is detectable by the sometimes complex and diagnostic shapes of cells such as the Haversian osteocytes in bone and much more precisely by quantitative morphometric methods.

Gene expression

Gene expression is known to be much affected by nanotopography [9,11,14,17] and also by a variety of signals that can be chemically printed onto a surface. The work of Dalby has shown that both precise and less precise nanotopography (the latter is made by polymer demixing) cause considerable changes in gene expression in several cell types. It is clear that the changes follow a sequence of expressions as they do in controls but the sequences are not identical. This area of work opens the possibility of designing structures and scaffolds that will have a degree of control of gene expression. Effects of nanoscale sized objects on a surface altered production of the cytokines (IL-6 and IL-8) [25].

Why use nanoscale devices rather than microscale devices?

At first sight it might seem surprising that we obtain large responses from nanometric features rather than micrometric ones which are closer to the cell itself in scale. Both scales of feature do produce many effects in cells but the probable reason lies in the fact that a cell contacting a microscale feature is only likely to sample a small number of areas which can be compared whereas nanofeatures provide a large number of areas for comparison and much steeper gradients in feature character.

No evidence has been found that a single topographic or chemical nanofeature can affect a cell. There appears to be a threshold of an appreciable number of features, such as length of a cliff edge, per cell [10] to establish an adhesion on which the cell can spread. But bear in mind that cells adhere reasonably well to flat culture dishes and well to ultra-flat surfaces with less than 3nm high or deep features Marked changes in the adhesion of a surface only began to appear in that work when about15, 000 molecule of fibronectin were derivatized per average cell area, [26].

Gradient comparison. Length of actin strands and frequency of focal contact.

A related reason may be that much of the pathfinding by cells occurs at the tips of the lamellopodia or filopodia. The lengths of these structures rarely exceed one micron so that the cell is almost compelled to sample very small lengths possibly because mechanical forces will result in the cytoskeletal component tending to pull away from the plasma membrane. In order to extract information about the surface comparisons must be made between either different parts of the cells or of sample information received over very small time intervals as the cell moves. An analogous problem arises from the ability of cells to detect very small concentrations of a chemical where the statistical variation in concentration over a few microns is so noisy that no orientation would result. Signal comparison over small time differences could provide a much more physically plausible system. An efficient way of comparing lengths may be to compare mechanical forces within the cell It is also clear [27] that discontinuities as for sample at the intersection of two plane in a surface are regions of especial 'interest' for cells. These areas are usually associated with high adhesion for cells and are the modern explanation of the reason for roughening surfaces by abrasion. The length of cytoskeletal elements may have a considerable effect on the ways in which a cell reacts to topography since this will modify force and adhesion comparisons.

Topographic features, as mentioned, above can provide surfaces of high or low adhesion depending on shape, spacing and dimensions of the nanofeatures. Riehle has shown that cells can react to small scale rigid microstructure even though that structure is overlain by many micra thickness of a softer material. This strongly suggests that the cells are interacting mechanically with the topography, Thus results with nanotopography can probably be interpreted as mechanical effects.

Since cell contraction is stimulated both by mechanical forces applied to the cells [28] and by chemical stimuli [29] and since cytoskeletal arrangements are affected by nanotopography and microtopography as well as chemical stimuli [30] it seems possible that at least the three systems center on signal transduction to adhesion systems and organization of the cytoskeleton, Explanation of the effects on gene expression can at least in part be explained by tensional effects on chromosome packing [20] and accessibility of the genome for transcription.

CONCLUSIONS

Work on the biological effects of nanofeatures has proceeded very rapidly with the development of many good methods for making such surfaces and with the increasing

availability of replicas but much remains to be done or to be more thoroughly established. Many patterns of nanofeature and different sizes and shapes of the features need to be explored and many more cell types need to be examined before definite conclusions can be drawn. This review is directed towards pointing out that there are a multitude of research projects to be carried out.

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